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# Phytonanofabrication: Methodology and Factors Affecting Biosynthesis of Nanoparticles

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## Abstract

The greener way of producing silver nanoparticles is the easiest, cheapest and most efficient way of producing large-scale nanoparticles that have no adverse effect on the environment. The nanosynthesis using various methodologies and the biological synthesis of silver nanoparticles have been discussed in detail. Plant extracts have been known to be competent for the extracellular biosynthesis of silver nanoparticles suggested by the various publications. Further, effects of various sources and methods on nanoparticle synthesis have been examined. Additionally, the impact of conditions such as dark, light, heating, boiling, sonication, autoclave on the size and shape of colloidal nanoparticles has been analyzed. Moreover, effects of specific parameters such as leaf extract concentration,  $\text{AgNO}_3$ , reaction temperature, pH, light and stirring time for nanoparticle synthesis are discussed, and the impact of silver nanoparticles on plant physiology has examined.

**Keywords:** silver nanoparticles, plant extract, biological nanoparticle synthesis, reducing and capping agents

## 1. Introduction

Nanotechnology involves learning how to formulate a really Tiny substance, perhaps through a bottom-up or a top-down approach. The former bottom-up strategy uses the active participation of both the reducing and the capping agent to somehow normalize metallic salt in the creation of metallic nanoparticles. The actual entities that taken part could be electrons present in the functional group of the reducing/capping agent and the electrons of metallic ions, for example, silver salt. In such an instance, the electron is available in the reduction agent's outer shell that mostly reduces unstable silver or other metallic ions to form zero-valent stable silver or metallic nanoparticles. Another method is the top-down approach, where a certain bulk material structure is imposed under multiple external forces, strain, stress, physical pressure, tension, temperature, using devices, techniques, equipment and instruments that fracture bulk material into tiny nanosized nanorange particles [1].

## 2. Nanosynthesis

In nanosynthesis, the reactant A (metallic salts) interacts with the reactant B (capping agent) where the nucleation reaction occurs. The entire phenomenon occurs at a specific temperature, and the nanoparticle synthesis is significantly improved by the stabilizing (agent) to form metallic nanoparticles and by-products. In several cases, the by-product or larger reaction structures may lead to a mass agglomeration, which suggests aggregation by chemical bonding of larger particles. However, the reacting metallic salts and the capping agent form nanoparticles that have been stabilized by a capping agent that directly prevents mass synthesis.

### 2.1 Types of nanoparticle preparation

#### 2.1.1 Physical preparation method

The physical method uses a “top-down” approach in which the bulk material is pulverized into fine particulate matter by means of force applied, such as crushing, impact, disruption, degradation, cutting, cryo-grinding, grinding, processing and homogenization. Physical methods use the milling process in micro-particle fracturing, and few examples involve ball milling, high-energy ball milling (HEBM), grinding, cryo-grinding, refining and homogenization: high process homogenization (HPH) and medium-pressure homogenization (UHPH). These physical productions are versatile methods that manufacture nanoparticles of larger size, diameter and volume, which are still controlled, generate surface defects, contamination, costly and time-consuming. It may also be possible to produce nanoparticles of the same size, but instrument assembly is very expensive and maintenance is too costly. This imperfection in the form of efficient and expensive maintenance culminated in the space travel of biological sources and methods for the nanoparticle manufacturing process [2]. The synthesis of nanoparticles using biological methods includes the synthesis of nanoparticles using living things or matter. There are several other ways to synthesize nanoparticles, such as chemical and biological methods.

#### 2.1.2 Chemical preparation method

The chemical preparation process involves a bottom-up technique and is the most frequently employed method for preparing silver nanoparticles in water or organic solvents as a stable, colloidal dispersion. Various chemical preparation methods are available, such as sol system, chemical preparations: CdS base, CdS/HgS/CdS composite, sol-gel process, hydrothermal, pyrolysis and chemical vapor deposition (CVD) process [3] (metallic) silver salt to produce nanoparticles. Here, commonly used reductants are borohydride, citrate, ascorbate and elemental hydrogen that reduce (metallic) silver salt to produce nanoparticles. Chemically synthesized nanoparticles are made of Cu, Ag, Au, Gold, etc., although the use of toxic chemicals in the synthesis protocol cannot be avoided. Alternative methods for nanoparticle synthesis are therefore explored in order to minimize the use of chemicals, and several physical methods were developed and implemented for synthesis of nanoparticles to resolve the chemical preparation.

#### 2.1.3 Biological preparation method

Physical methods are expensive and require strong material, toughness, strength, etc. for nanoparticle synthesis, which may not be suitable for large-scale

Sr. no.	Plant species	Plant part	NP size	References
1	<i>Aloe vera</i>	Leaves	50–350 nm	[5]
2	<i>Argyrea nervosa</i>	Seeds	20–50 nm	[6]
3	<i>Acorus calamus</i>	Rhizome	31.83	[7]
4	<i>Allium sativum</i>	Sucrose and fructose	4–22 nm, 4 4 ± 1.5 nm	[8]
5	<i>Boerhaavia diffusa</i>	Whole plant	25 nm	[9]
6	<i>Citrus sinensis</i>	Peel	10–35 nm	[10]
7	<i>Cocos nucifera</i>	Inflorescence	22 nm	[11]
8	<i>Calotropis procera</i>	Plant	19–45 nm	[12]
9	Olive extract	1 ml	30 nm	[13]
10	<i>Passiflora foetida</i>	Leaf disc	14 nm	[14]
11	<i>Terminalia chebula</i>	Fruit extract	100 nm	[15]
12	<i>Thevetia peruviana</i>	Latex	10–60 nm	[16]

**Table 1.**  
Reports of silver nanoparticle synthesis using plant species, biomolecules, nanoparticle sizes and references [17].

production of nanoparticles. Although chemical methods are used in the mass production of nanoparticles, the key product generated in the synthesis of nanoparticles tends to cause pollution that ultimately poses a risk to life in terrestrial and aquatic life, leading to environmental accumulation. Physical and chemical methods therefore constitute invaluable processes, while the problem can be overcome by using a biological solution that uses living organisms, bacteria, plants, etc., which is sometimes called the green method. These green strategies include plants, microorganisms and enzymes that are considered to be nonhazardous to humans through the use of natural biological agents that are ecofriendly, biodegradable as reducing, capping and stabilizing agents for nanoparticle synthesis. These biological preparations require a living origin, such as insects, parts of plants, microorganism such as *F. oxysporum*, enzymes, amino acid, vitamin polysaccharides, polyphenols, amino acid, tea, coffee, wine, winery waste, banana, red grape, table sugar and glucose are used for capping and stabilizing. Plant components for the manufacture of nanoparticles have significant advantages over other methods such as easy preparation, processing, cost-effective, rapid development, reproducible, stable components, environmentally friendly, avoiding the use of harsh and toxic chemicals and zero contamination of the environment [4]. Nevertheless, plant-based preparation has become common due to its simple, comprehensive availability and extensive range of samples, including plant part extracts and natural products. In addition, plant, leaf core, root, latex, seed and stem contain polyphenols and have been successfully used for metal particle synthesis [4]. Moreover, plant extract, more specific leaf extract, seed extract, stem extract, fruit extract, root extract and flower extract are used for nanoparticle synthesis. **Table 1** displays many studies of plants mediated silver nanoparticles synthesis reports with their reducing biomolecules and the respective nanoparticle size.

### 3. Nanofabrication using plant sample

The biological method that uses plant samples is the easiest and cheapest way to use a bottom-up approach to nanoparticle synthesis. In broad aspect, there are large

number of research papers representing other plant parts for nanoformulation, which includes callus, seed, fruit, stem, flower, tendril and root, which we termed as green synthesis because the initial as well as the byproduct after nanoparticle synthesis both are ecofriendly and biodegradable. This greener method of plant-derived reducing agent is used to reduce silver ions to form stable zero-valent silver called silver nanoparticles. Likewise, other metallic nanoparticles, such as gold, copper, silver, titanium, etc., are used for synthesis of nanoparticles using plant extract. The basic synthesis of nanoparticles using plant extract and silver salts was discussed in detail in the following paragraphs. Nanoparticle synthesis is a single pot reaction in which the preparation of the plant extract,  $\text{AgNO}_3$ , is performed and these solutions are combined to form silver nanoparticles [14]. Silver salt was usually used in this chapter for analysis along with plant extract for the formulation of nanoparticles.

### 3.1 Preparation of plant leaf extract

In general, the specific amount of the plant sample is determined in grams for the preparation of the extract in solvents, and the extract filtrate is diluted with sterile distilled water. The diluted filtrate is then used as a reducing agent for silver salts to form silver nanoparticles. The general procedure for the preparation of the extract is as follows: first, the 10 g leaf of *Passiflora foetida* washed with tap water and dried at room temperature. Second, washed dried leaf specimens chopped into small pieces and macerated directly into 75 ml of autoclaved distilled water. Eventually, the mixture is centrifuged at 4000 rpm for 10 min and filtered with the aid of muslin cloth or syringe filters (stored at 4 °C for future use). **Figure 1** shows the step-wise process of preparation of leaf extract by boiling method for the synthesis of silver nanoparticles [18]. As we are dealing with nanoformulation, it is very important to use the (diluted) plant extract at a minimum to reduce the amount of silver salt.

### 3.2 Preparation of $\text{AgNO}_3$ solution

Similar to plant extract, the amount of silver salt must be low and precise in order to form stable nanoparticles. If the reaction of silver salt is more or more in bulk, the synthesis of nanoparticles may be hindered. For this purpose, the minimum quantity of reacting material is used for the effective, slow, nucleation of nanoparticles. Because silver nitrate ( $\text{AgNO}_3$ ) is very expensive, it is important to



**Figure 1.** Step-wise process of preparation of leaf extract of *Passiflora foetida* by boiling method for the synthesis of silver nanoparticles [18].



measure it in a very small quantity (1 mM) and therefore a stock of 100 mM aqueous silver nitrate solution ( $\text{AgNO}_3$ ) is prepared and used to optimize the synthesis of silver nanoparticles.

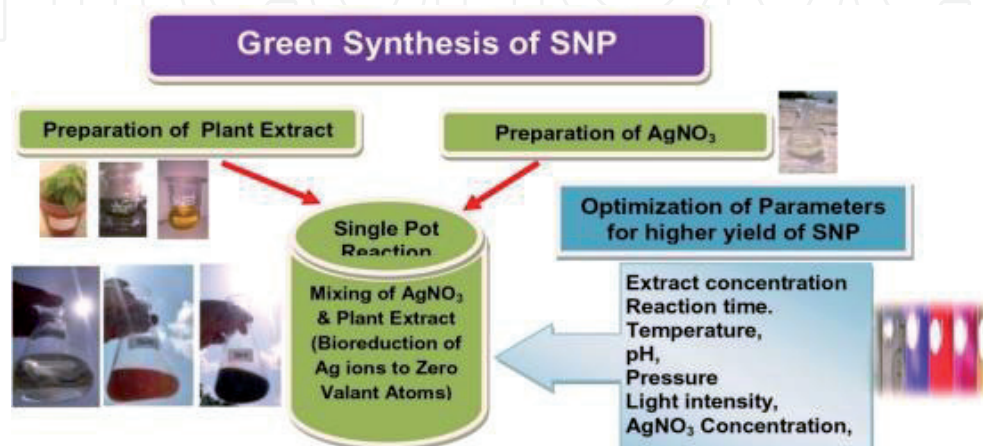
### 3.3 Nanoparticle synthesis

Nanoparticles are easily synthesized by combining the appropriate concentration of plant extract and silver salt, and in short, 1 mM of silver nitrate (95 ml) is mixed with 5 ml of (diluted) leaf extract and kept at constant stirring in the 250 ml round bottom flask on a magnetic stirrer at room temperature. Later, solutions are allowed to react with each other in order to form nanoparticles. Synthesis of silver nanoparticles is confirmed by a change in the color of the solution from light green to dark brown, detected clearly by the naked eye (visual observation). This noticeable change in color is the primary indicator for the synthesis of Ag nanoparticles. The general optimization method for nanoparticle synthesis is shown in diagrammatic form in **Figure 2**.

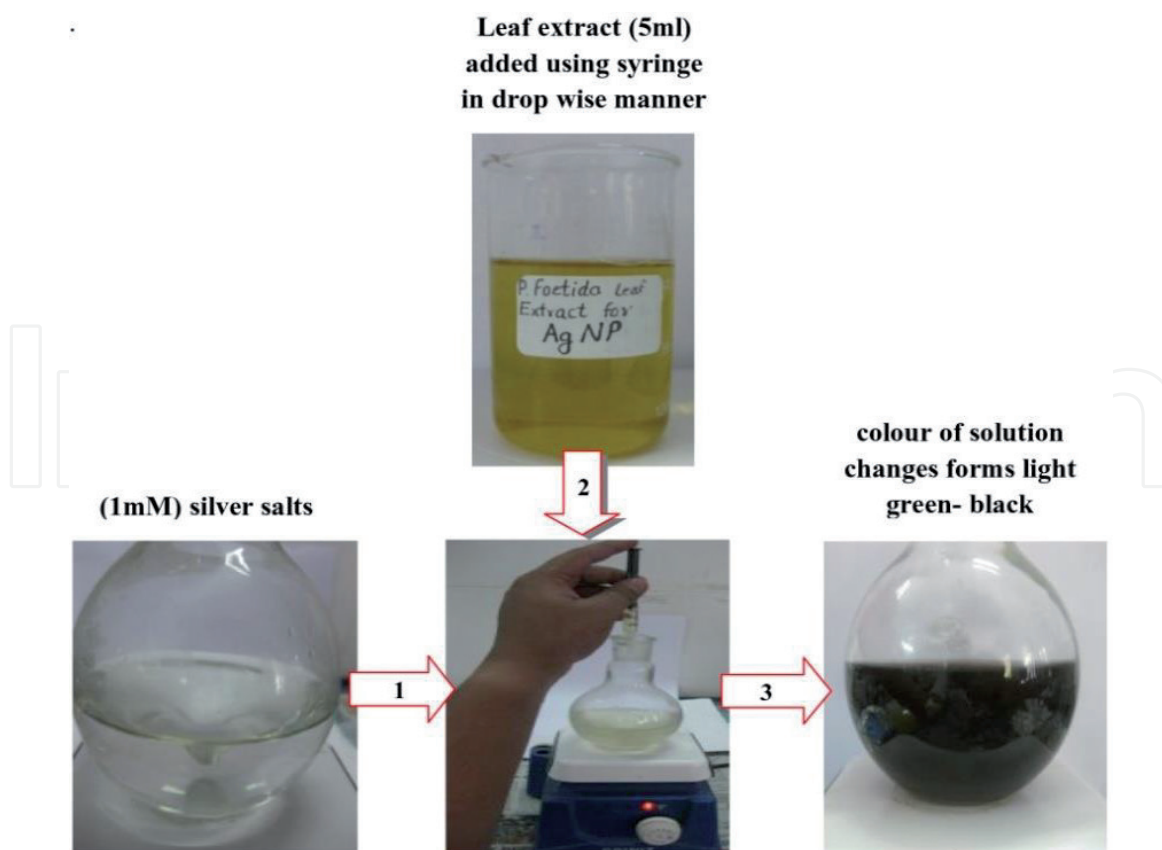
Unless, after some time, the mixture does not show any change in color, it would be due to the acidity of the medium. It has been found in several papers that the alkaline solution is favorable to nanoparticle synthesis. Thus, 0.1 mM of NaOH is therefore used to produce a mixture solution into alkaline. It is vital that either the silver salt or the extract can be applied gradually using a syringe. The release of a moderate volume of extract into silver salt requires the proper synthesis of silver nanoparticles. The complete process of green synthesized silver nanoparticles at room temperature is shown in a diagrammatic form in **Figure 3**.

### 3.4 Nature of silver nanoparticles

Due to their application intent, colloidal nature of the synthesized silver nanoparticles is a critical aspect to consider. If the silver nanoparticles are not stabilized, they become heavy and will be found at bottom of flask or beaker. This aggregation problems can be resolved by sonicating the silver nanoparticle solution as well as using NaCl salt which hides the charges allowing the particles to clump together to form aggregates. Generally, a peak around 420–460 nm confirms stable silver nanoparticle synthesis, while silver nanoparticle aggregates form a broad peak around 350–525 nm with a decrease in the intensity of the plasmon absorbance [19].



**Figure 2.** General optimization method, criteria, instrument characterization and potential applications of synthesized nanoparticles [18].



**Figure 3.**  
Complete process of green synthesis of silver nanoparticles at room temperature [18].

## 4. Nanoparticle synthesis methods and conditions

To nanotechnologists, nanoparticle formulation is being the most important aspect, where they use biological methods specifically plant extract to reduce metal ions and this will be used in further detail. The synthesis of nanoparticles is carried out using various methods such as microwave oven, autoclave, sonication, heating, boiling, etc., as well as under different conditions such as dark, daylight, light color different wavelength, etc. Likewise, the synthesis of nanoparticles by different methods implies the effect of specific system parameters such as light, heat, temperature, pressure, etc. on the kinetics of electrons present during nanoparticle synthesis in silver salts and capping agents. In comparison, the synthesis of nanoparticles in particular conditions indicates the effect of the conditions available on the synthesis of nanoparticles.

### 4.1 General synthesis process

In brief, the reactant, that is, plant extract and the  $\text{AgNO}_3$  (1 mM) are mixed in a specific ratio such as 95:5 ml, respectively, and the reaction is conducted under specific instrument or machine, such as early mention of microwave oven, autoclave, sonication, heating, boiling, etc. During a study of these methods or conditions, the size or shape and type or form of nanoparticles formulated depend upon the kinetics of reaction that relays on the adopted methodology. In this section, we addressed different conditions under which nanoparticles could be generated. For example, the effect on the synthesis of nanoparticles of a specific sound wave may differ from that of light intensity or color. The sunlight has a specific effect on nanoparticle synthesis, which synthesizes nanoparticles of different sizes and shapes.

## 4.2 Various methods

### 4.2.1 Microwave oven

The microwave oven that could be used for synthesis of nanoparticles is one of the popular techniques. The microwave oven is a method for the processing of nanoparticles using different intensities of microwave radiation. Here the reactant, which may be a capping agent, is mixed with metallic salts and the mixture is placed in a microwave oven. Microwave treatment is administered to the reaction mixture in a short burst of 20 s for 5 min, which has influenced the kinetics of the mixture and drastically alters the size and shape of the nanoparticles. The radio wave in microwave oven is utilized to activate compounds or secondary metabolites in extract to reduce metal salts. Radio wave at a specific frequency is absorbed by extract components (phytochemicals and secondary metabolites) or activates certain extract enzymes to reduce metallic salts for stable nanoparticle production. As a result, microwave oven-assisted silver nanoparticles form in less time and are therefore the fastest way to synthesize [18].

### 4.2.2 Autoclave

The basic instrument used in the laboratory is an autoclave that produces steam at a pressure of 50 psi and reaches a temperature of  $\sim 121^{\circ}\text{C}$ , at which organisms, pathogens and even endospores are killed or destroyed within 15 min. However, nanotechnologists use autoclaves to create a specific pressure and temperature for nanoparticle synthesis. In this process, kinetic of mixture is influenced by autoclave which is preheated for 5 min, once the pressure rises the flap is opened and the mixture flask or round bottom flask is kept in it for 5 min. Autoclave is not allowed to achieve 50 psi, instead, the autoclave is preheated to 5–10 psi for nanoparticle synthesis, and capping agent and the reducing agent are mixed with metallic salt and kept inside the autoclave for a specific time. Care must be taken in this method, as the autoclave is a very simple but dangerous machine, if the pressure rises above the crucial level and when it is opened critically results in steam or blast. Similarly, sonication is another way that could be used to synthesize nanoparticles [18].

### 4.2.3 Sonication

Sonication produces excitation of compounds from various biological sources, such as plants, microbes, animals, extracts, etc. It uses sound energy to agitate metallic salt in a reaction mixture ( $\text{AgNO}_3$  + plant extract) to form nanoparticles. The specific wave hertz (Hz) is adjusted and applied to the reaction mixture for a specific time and the synthesized silver nanoparticles can be easily collected. Typically, an ultrasonic frequency of 20 kHz is administered to the reaction mixture. It is also referred to as ultrasound used for the synthesis of nanoparticles, nanoemulsions, nanocrystals and other forms. In addition, sonication is used for dispensing nanoparticles in liquid solution to disaggregate larger nanoparticles. Similar to sonication heating, the reaction mixture could also be another method for synthesis of nanoparticles [18].

### 4.2.4 Heating

In the heating process, heat energy is transferred to and from the mixture of extract and silver salt. Thus, when the reactants are heated, the heat excites the electron of the reacting secondary metabolites as well as the  $\text{Ag}^{2+}$  ions, the  $\text{NO}_3^{2-}$  ion silver salts to reduce  $\text{Ag}^{2+}$  ions to form Ag zero valent. In specific, the reaction



mixture of metallic salt and plant extract is heated to a burner for particular temperature (10–100 °C) for certain period of time until the solution boils. Care must be taken not to overheat the reaction mixture as it comes out of the bottle. After boiling, the mixture of the solution may cool down at room temperature. To confirm that the synthesis of nanoparticles has been completed, the solution is observed to change the color from light green to dark brown. It may be possible to notice a change of color within minutes or it may take hours a day for a change of color depending on the reducing and capping agent involved in the process [18].

#### 4.2.5 Boiling

The boiling method could be an approach in which the heat is given up to the boiling point in order to transform the liquid sample into a vapor state. Phytochemicals, secondary metabolites as well as metallic salt reactants are activated by boiling. The free electron of the secondary metabolite or phytochemicals reacts with metallic salt and helps to maintain stability. The reaction mixture of metallic salt  $\text{AgNO}_3$  and plant extract is boiled on a burner or gas in a beaker for 5–10 min, the boiled solution that come out of a beaker, so care must be taken and the beaker is put out of a gas or a burner so that it may not falls out of a beaker. Once the solution boils, allow the mixture solution to cool down to reach room temperature and the color change from light green to dark brown is observed for verification of the synthesized nanoparticles [18].

### 4.3 Various conditions

The general condition that can be used in a laboratory is discussed in the following paragraph. In general, light of different wavelengths activates the enzyme and activated caused the reduction of silver or metal salts to the formation of nanoparticles and by-products. In this section, light-induced nanoparticle systems have been discussed.

#### 4.3.1 Dark condition

Until going to light condition, it is very important to understand the effect of the dark condition on nanoparticle synthesis. Thus, the reaction mixture (extract +  $\text{AgNO}_3$ ) is kept in a dark state. The nanoparticle synthesis reaction could be performed in dark where the conical flask is covered with aluminum foil to confer the darkness. Originally, the drops of  $\text{AgNO}_3$  (1 mM) 5 ml are continuously applied to the known concentration of leaf extracts of a specific plant (*Passiflora foetida*) 95 ml in conical flask. Such mixtures are kept at continuous stirring using a magnetic stirrer. The reaction must be conducted under dark conditions as light oxidized metal, resulting in photo-leaching due to its photo-reactivity properties. Some studies have indicated that synergistic antimicrobial activity is not linked to photo-activity. Nevertheless, the dark state of treated nanoparticles was found to be more stable than UV-light-synthesized particles [14, 18]. The [20] analysis is of synergistic bactericidal activity of silver titanium oxide ( $\text{AgTiO}_2$ ) nanoparticles in both light and dark conditions. Therefore, the synthesis of nanoparticles using dark could be a convenient method in certain areas of application.

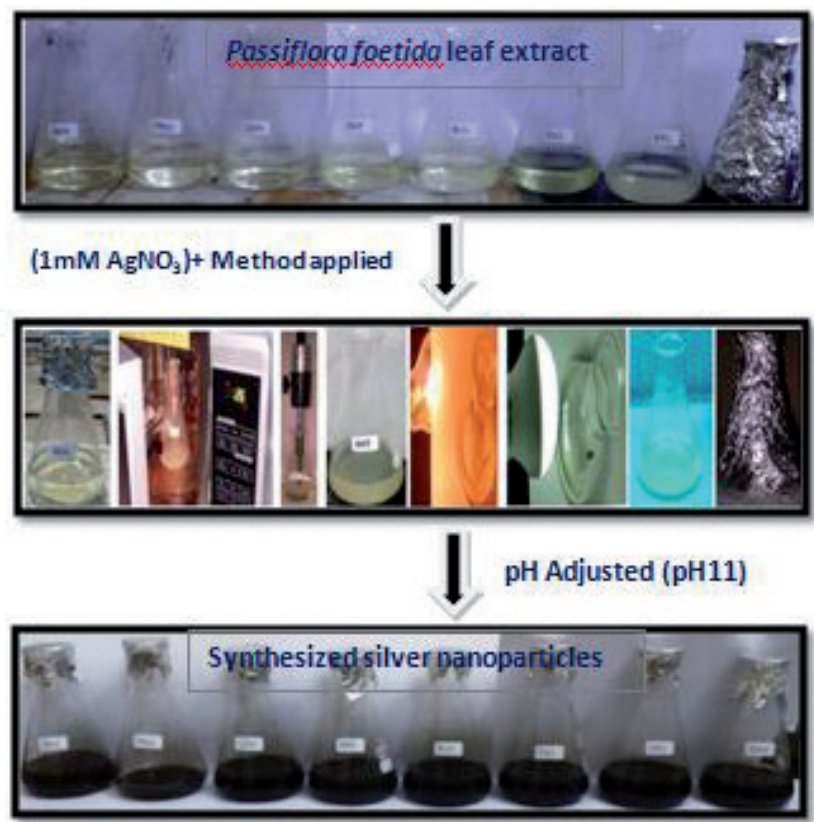
#### 4.3.2 Sun light conditions

Corresponding to the dark environment, when the reaction mixture (extract + silver salt) is maintained under sunlight conditions, it may activate certain

enzymes (phytochemicals and secondary metabolites) present in the leaf extract and express different results that are comparable to the reactions performed at a different condition/room temperature. There are several publications explaining the light-induced nanoparticle synthesis of silver, gold, titanium, aluminum, etc. The sunbeam emits electromagnetic energy in a wide range of wavelengths that enhances, activates or induces the kinetic reaction of silver salts with the functional group of the reacting secondary metabolites or phytochemicals present in the plant extract. Nanoparticles produced by sunlight are produced in much less time and are found to be more stable than room temperature and other conditions [14]. Apart from daylight, the other wavelength light could also be used for nanoparticle synthesis [18].

4.3.3 Light color (wavelength)

The light of different colors could produce different sizes and shapes of nanoparticles. Red, green, and blue are the primary colors of light that are used in different conditions in different ratios to create certain light colors. It is noted that silver nanoparticles under different light colors, such as green, blue, red, yellow and orange 15 W, result in silver nanoparticle synthesis where the blue color light of 15 W shows good UV absorption at 400 nm compared to other color lights. Therefore, the size and shape as well as the stability of nanoparticles could be altered by different wavelengths of light. Like the various wavelength of light, we would like to explore the idea of the influence of sound on nanoparticles [18]. **Figure 4** shows the silver nanoparticle synthesis using various methods and conformation by visual change in colors.



**Figure 4.** Synthesis of silver nanoparticles using various methods/conditions: sun, microwave oven, sonication, autoclave, yellow bulb, white bulb, UV light and dark (from left to right) [18].

4.3.4 Influence of sound

In this process, the protocol is the same, and it is the reaction process carried under different conditions using selective instruments. The sound waves consist of longitudinal waves and transverse waves in solids that can be easily produced using a sound source such as the vibrating diaphragm of a stereo speaker. Therefore, the noise or acoustic environment is used to study different aspects of nanoparticles. Sound waves are used to isolate biological nanoparticles from bio-liquid samples. However, sound effects on the synthesis of bio-nanoparticles have not been studied. So, we are trying to provide some insight on the impact of sound on bio-nanoparticle synthesis. We are very eager to know the effect of sound including (jazz/rock/gaze/pop song) and the level of sound frequency on the synthesis of silver nanoparticles. Once the nanoparticles are synthesized, they are used for characterization by means of the available instruments, which will define nanoparticles size, shape, surface load, aggregates, colloidal, capping agent detection, reducing agent confirmation and conjugation (its ability to combine) with another polymer [18]. **Table 2** indicates the possible conditions under which the manufacture of silver nanoparticles is feasible.

4.3.5 Novel concepts

The novel concept could be traced out where different strategies can be applied to nanoparticle synthesis. It is reported in [14] paper that a specific type of wound stress has been applied to the plant leaf sample of the medicinal plant *Passiflora foetida* (leaf cut 2 cm in diameter and used to collect diffusate) and that secondary metabolite-induced stress has been extracted (see **Figure 5**). In this context, a stressed-induced plant extract is used, which contains stress-induced secondary metabolites and phytochemicals that reduce silver ion and act as a capping agent responsible for the synthesis of silver nanoparticles. The use of such stress-induced pool extracts may be considered for the formulation of silver nanoparticles [14].

Sr. no	Method/conditions	Point to note	Other remarks
1.	Dark condition	Light oxidized silver and leads to photo leaching	May cover with an aluminum foil
2.	Sunlight	Activate some enzyme present in leaf extract	Yields best results, Produced SNP quickly
3.	Domesticated microwave oven	Short burst of 20 s for 5 min to reaction mixture	Effects on SNP size and shape
4.	Autoclave	Preheating for 5 min once the pressure rises, the flap is opened and the mixture flask is kept in it for 5 min	If the pressure rises above the crucial level, opening it at that point may be critical
5.	Sonication	120 Hz of wave is adjusted and applied on reaction mixture for particular time	Varying Hz results in varied size and shape of SNP
6.	Light color	Green, blur, red, yellow and orange of 15 W	Watts could be changed for making various sizes/shapes of SNP
7.	Influence of sound	(Jazz/rock/gazel/pop song)	Volume level high/low may produce unique SNP

*Note: The type or form of silver nanoparticles (SNPs) formulated depends upon the kinetics of reaction that relays on the adopted methodology.*

**Table 2.**  
Possible conditions under which the manufacture of silver nanoparticles is feasible [18].



**Figure 5.**  
 Leaf disc preparation using test tube for applying wound stress to *P. foetida* and synthesis of silver nanoparticles using leaf disc under sunlight [14].

## 5. Factors affecting biosynthesis of nanoparticles

Manufacturing nanoparticles is a very critical process where the environmental factor is temperature and pressure; light intensity plays an important role in the synthesis of nanoparticles. Parameters such as sample leaf extract concentration,  $\text{AgNO}_3$  concentration, reaction temperature, reaction time, pH reaction and different light reactions play a key role in the creation of silver nanoparticles of varying size, shape and dimension. Similarly, the light conditions such as sun light, blue light, red light, bulb light, tube light and in dark devoid of light effects are examined for nanoparticle biosynthesis. Several parameters that play a crucial role in the synthesis of the particular nanoparticles required have been addressed briefly. Such parameters are modified in order to produce the desired size of nanoparticles for the study of specific activity. Temperature is one of the most important parameters for nanoparticle synthesis.

### 5.1 Reaction temperature

Temperature has a specific effect on the process of synthesis of silver nanoparticles. Usually, the reaction is performed at room temperature, which takes a long time to complete, but can be accelerated by increasing the temperature of the reaction mixture. The temperature can be adjusted to between 30 and 100°C. Increased reaction temperature led to a rapid reduction in the rate of  $\text{Ag}^+$  ions and subsequent homogeneous nucleation of silver nuclei, enabling the development of small size silver nanoparticles [18]. It has been found that as the temperature of the reaction mixture increases the rate of nanoparticle synthesis decreases, as well as the stability increases. In addition, silver nanoparticles synthesized at higher temperatures have small nanoparticle sizes. Another parameter that is important during silver nanoparticle synthesis is volume of leaf extract.

### 5.2 Volume of leaf extract

The quantity of leaf extract, that is, the volume of leaf extract, also affects the processing of nanoparticles and affects the time required for silver nanoparticle formation. Because leaf extracts are a major part of the reduction of silver ion, their volume up to a certain quantity is efficient in the formation of silver nanoparticles. Generally, the 1, 5, 10, 15, 20 and 100 ml leaf extract could be checked for effect. It is advised to use 1, 2, 3, 4 and 5 ml, since less volume is required for nanoscale particle synthesis. The average particle size of the synthesized silver nanoparticles is highly influenced by the concentration of leaf extract that the contents (phenol, polyphenols, polysaccharides, tannins and anthocyanins) contributes significantly



to the reduction of  $\text{Ag}^{++}$  ions to the formation of stable Ag atoms in nanoscale, making it the main parameter during optimization for nanoparticle manufacturing.

### **5.3 Effect of pH**

A major influence of the reaction (pH) is its ability to change the electrical loads of biomolecules, which may affect their ability to cap and stabilize and, consequently, the growth of nanoparticles [21]. In general, in the course of research work experimentation, our observations suggest that, if the pH of the reactant is acidic, the transition from light green to dark brown will take a much longer time, indicating that the acidic medium is not appropriate for nanoparticle synthesis. The size of nanoparticles is expected to be higher in the acidic medium than in the basic media, which is also reported in different studies. It was our observation that particle sizes at pH 3 were greater than those at pH 8 with regular spherical shape, both of which were observed in the TEM analysis. The alkaline pH condition facilitated the reduction and stabilizing capacity of antioxidants in the leaf extract. During optimization, the pH spectrum could be tested from 3 to 11 pH and potential spectroscopic UV tests could detect changes in absorption.

### **5.4 Silver nitrate ( $\text{AgNO}_3$ ) concentration**

The concentration of  $\text{AgNO}_3$  is measured in a range of 0.1–1 mM or even more concentrations, such as 10, 50 and 100 mM [22]. However, 1 mM concentration is best studied and suggested as we are actually synthesizing nanoparticles that are very minute and cannot be seen with naked eyes. Therefore, a very small quantity of the reactant is required for the reaction to occur. If the concentration of the reactant is increased, the reduction of  $\text{Ag}^{++}$  will not be successful and the accumulation could be noticeable. Actually, it does not make sense to use higher concentration for synthesis of nanoscale particles. Another factor vital for synthesis is the reaction stirring time for up to which reaction mixture is stirred.

### **5.5 Reaction stirring time**

The reaction stirring time is the time required for silver nanoparticle synthesis starting from the reactant is added in the beaker to occur reaction. The stirring time will enable the proper interaction of silver salt with the reducing complex components present in test leaf extract. The plant containing the more secondary metabolites or phytochemicals will reduce the silver salt in less time, in other terms the plant containing fewer reduced compounds will take longer time for reducing silver salt. However, the less number of secondary metabolites reduces silver salt and nanoparticles formed quickly in very less time. The stirring time will be dependent on reaction mixture acidity, basicity, temperature, reducing power of extract, light intensity, enzyme and secondary metabolites of the test plant extract. Ref. [23] concluded that the stability of silver nanoparticle synthesis using glutathione as a reducing agent increases at 72 h and confirms the visible UV absorbance at 344–354 nm. Thus stirring time affects the synthesis of nanoparticles, the duration of the synthesis of nanoparticles, the time allowed for the interaction of silver nitrate and leaf extract.

## **6. Fate of nanoparticles in plants**

Targeted application nanoparticles have led to their use in many areas, including medical, pharmacological, chemical, paint, fertilizer, geosensing, agriculture, etc.

Nonetheless, nanoparticles are very durable and not easily degraded, which may remain in nature for decades or more. They may therefore remain in the climate, soil, air, water and eventually accumulate in the food chain. As a result, potential fates of nanoparticles in microorganisms, insects, animals, humans, plants and the atmosphere are very important.

### **6.1 Nanoparticles' effect on plants**

Plants are faced with nanoparticles due to application in the field of plant protection. These nanoparticles are absorbed or collected or absorbed through the cell wall, the leaf surfaces, and the stomata or through the root from the soil. Once entry into the plant through the leaf surfaces, cell cytoplasm, mitochondria, ribosome, plant proteins, and enzymes radically alter their normal functions that cause cell death. They also interact with different processes in cells that cause alteration in phytohormones, metabolites, photosynthesis, transport and apoptosis-inducing metabolism. In addition, nanoparticles binding induce oxidative stress leading to degradation of proteins, lipids, nucleic acid, stress-related genes and increased antioxidant development for ROS activity, effects cell function and leads the oxidation of proteins, lipids and nucleic acid [24]. The nanoparticles could activate certain cells that activate apoptosis by intrinsic pathway or necrosis. Thus, while formulating the nanoparticles or the nanoparticles-based products for various applications, it is very important to keep the track of nanoparticle products side effect into environment. These nanoparticles interact through an unknown mechanism and sometimes act in support of and increase the growth of seeds and plants. Nanoparticles, on the other hand, could inhibit seed and plant growth without any clues. Nanobiopesticide (NBP) products must therefore be organic and nontoxic and their final deposition in plants must be studied exclusively. There are several studies that use nanoparticles or nanobiopesticides to combat pests; on the other hand, these nanobiopesticides are being purged into healthy plant cells. Therefore, the pesticide in nanoform or nanomaterial must be eco-friendly to plants and, at the same time, the NBP must act selectively to suppress pests or insects.

### **7. In vitro assay**

The potential prospectus on the capacity of nanoparticles is tested via in vitro assay that based on Kirby-Bauer technique. The inherent potential of nanoparticles is used on various applications in laboratory as well as in industries. There are various ways to trace the inhibition property of silver nanoparticles. The minimum inhibition concentration of dried powder nanoparticles is important to determine the dose of nanoparticles against pathogens, and the zone of inhibition by silver nanoparticles is compared with the standard antibiotics. The concentration at which silver nanoparticles shows some effect to the microbe or fungi is determined by several methods such as agar well diffusion, disc diffusion and toxicity assays that are generally used for detecting the minimum concentration of silver nanoparticles for its antimicrobial/antifungal activity, larvicidal activity, anti-inflammatory, anti-platelet activity, anti-angiogenesis, antiviral, antilarva and pupicidal activity [25].

### **8. Applications**

Nanotechnology has given nanomaterials a chance to be used in different fields. Thanks to their nanosize and stability, there are countless applications for nanoscale

particles. Nanoparticles in nanoscale have a positive effect in their application in different fields, their modification at nanolevel in order to modify their properties and function to the advantage of food industries as well. The commercial ways have been taken up by the nanotechnology in food packing and processing. This technology has made possible to improve clinical clothing, plastic material, ceramic, surface disinfectant, dentistry purpose, virus, bacteria control, antimicrobial paints, textile, air disinfectant, waste water treatment, food preservation, agriculture application as pest, insect, larva, pupa control as nanoparticles pesticide (NPP), nanobiopesticides (NBP), nanofiler, nanofertilizer, etc. More than this, the plasma process is used in plasma farming, advanced packaging, labeling, sterilization and for food production applications. Therefore, the application of nanoparticles to artificial intelligence and robotics will contribute to the future of specific material benefit control for economic development.

## **9. Conclusion**

Silver nanoparticle fabrication is a single pot reaction that transforms water soluble components such as  $\text{AgNO}_3$ , plant-based capping and stabilizing agent (secondary metabolites/phytochemicals) into water-insoluble components, that is, nanoparticles. Biological methods are the best methods due to their advantages such as low cost preparation, avoidance of environmental contamination, nontoxic by-products and adequate supply of samples, which can be scaled up for large production. Various conditions, such as dark, sunlight, microwave oven, autoclave, sonication, different wavelengths of light, heating, boiling, etc., and various other sources, methods, may apply as required by experiments. In addition, conditions such as dark, sun, heating, boiling, sonication and autoclave can be checked for the size and shape of colloidal nanoparticles. The stability of nanoparticles depends on different parameters such as leaf extract concentration,  $\text{AgNO}_3$ , reaction temperature, pH, light and stirring time to optimize and determine the size and shape of nanoparticles. Potential nanoparticles have been applied in many cross-disciplinary fields, from agriculture to medicine, but their effect, fate and accumulation on plants remain a mystery.

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## **Conflict of interest**

The authors declare no conflict of interest.

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